

ISOLATION AND IDENTIFICATION OF BACTERIA CAPABLE OF SULFATE  
REDUCTION FROM PALM OIL SLUDGE

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*To all my beloved family members;  
my lovely husband, Omid  
and my kind child Ali*

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## ABSTRACT

Sulfur in its native is a yellow crystalline solid. In nature, it occurs as the pure element or as sulfide and sulfate minerals. Inorganic sulfur compounds can be found in the form of sulfate, sulfide, sulfite, thiosulfate, elemental sulfur and polythionates. Sulfate appears to be the most stable and abundant form of sulfur available for use by living organism in the biosphere. This present study focused on the isolation and identification of bacteria capable of sulfate reduction from palm oil sludge (POS). POS is one of the most difficult and complex industrial waste produced in Malaysia from palm oil processing plants. Three different samples of POS were collected from different pond of Palm Oil effluent in palm oil processing plant in Sedenak, Kulai, Malaysia. The concentration of sulfate content in the samples were analysed in order to determine the sample that contain high population of bacteria capable of sulfate reduction. This ensures the possibility of isolating the bacteria of interest from the selected sample. Results from SRB-Bart kit analysis showed that POS from raw pond contain high population of SRB or related bacteria and was chosen for further isolation of the bacteria. Isolation of the bacteria was conducted using selective enrichment method followed by growth on solid medium using rolling bottle technique. The isolation has successfully separated five different pure culture coded X2 , X1, E, B and C that were further identified using the analysis of amplified 16S rRNA sequences of the individual bacterium. Four of the bacteria namely E, B, X1, and X2 were found able to reductively degrade sulfate. These bacteria were able to grow and reduce limited amount of sulfate thus indicated to the assimilatory reduction of sulfate activity of these bacteria.

## ABSTRAK

Sulfur asli adalah pepejal kristal kuning. Secara semula jadi ia berlaku sebagai elemen tulen atau sebagai mineral sulfida dan sulfat. Sebatian sulfur bukan organik boleh didapati dalam bentuk sulfida sulfat, sulfit, thiosulfate, sulfur unsur dan polythionates. Sulfat merupakan bentuk yang paling stabil dan banyak didapati untuk digunakan oleh organisma hidup dalam biosfera. Kajian ini memberi tumpuan kepada pengasingan dan pengenalpastian bakteria yang mampu mengurangkan sulfat dari enapcemar kelapa sawit (POS). POS adalah salah satu sisa yang paling sukar dan kompleks oleh perindustrian yang dihasilkan di Malaysia dari kilang pemprosesan minyak sawit. Tiga sampel POS yang berbeza telah dikumpulkan dari kolam minyak sawit efluen yang berbeza di loji pemprosesan minyak sawit di Sedenak, Kulai, Malaysia. Kepekatan kandungan sulfat dalam sampel dianalisis untuk menentukan sampel yang mengandungi populasi bakteria yang mampu mengurangkan sulfat tertinggi. Hal ini bagi memastikan kemungkinan bakteria berfaedah dapat diisolasi daripada sampel yang dipilih. Keputusan dari SRB Bart kit analisis menunjukkan bahawa POS dari kolam mentah mengandungi populasi bakteria SRB yang tinggi dan telah dipilih untuk diisolasi bagi proses selanjutnya. Pengisolasian bakteria telah dijalankan menggunakan kaedah pengayaan terpilih diikuti oleh pertumbuhan pada medium pepejal menggunakan teknik botol *rolling*. Pengisolasian telah berjaya mengasingkan lima kultur tulen yang dikodkan sebagai X2, X1, E, B dan C yang seterusnya dikenal pasti menggunakan analisis 16S rRNA urutan bakteria individu. Empat bakteria iaitu E, B, X1, dan X2 telah didapati mampu untuk mengurangkan kandungan sulfat. Bakteria ini mampu untuk berkembangbiak dan mengurangkan jumlah sulfat dalam kadar terhad, lantas menunjukkan pengurangan asimilasi aktiviti sulfat bakteria ini.